

What is claimed:

1. A method to detect disease in a patient comprising:
digesting a phospholipid in a sample of bodily fluid from the subject with a first enzyme
5 to produce substrate;
reacting the substrate with a second enzyme in an enzymatic cycling reaction to produce
a detectable product;
determining the concentration of phospholipid by measuring the detectable product; and
correlating the concentration of phospholipid to the disease condition by comparison to a
10 normal concentration.

2. The method of claim 1, wherein said first enzyme is selected from the group
consisting of phospholipase B, lysophospholipase, phospholipase A₁, and phospholipase A₂.

3. The method of claim 1, wherein the second enzyme is selected from the group
consisting of glycerol-3-phosphate dehydrogenase, glycerol-3-phosphate oxidase, glycerokinase
and glycerol dehydrogenase.

4. The method of claim 1, wherein the substrate is glycerol-3-phosphate.

5. The method of claim 1, wherein the detectable product is hydrogen peroxide.

6. The method of claim 5, wherein the step of determining the concentration of phospholipid by measuring detectable product comprises measuring an increase in hydrogen peroxide by colorimetry.

5 7. The method of claim 1, wherein the detectable product is NADH.

8. The method of claim 7, wherein said step of determining the concentration of the phospholipid by measuring the detectable product comprises measuring oxidation of NADH.

10 9. The method of claim 1, wherein the step of reacting the substrate in an enzyme cycling reaction comprises reacting G-3-P with glycerol-3-phosphate dehydrogenase and glycerol-3-phosphate oxidase.

15 10. The method of claim 1, wherein, the sample of bodily fluid is selected from the group consisting of plasma, serum, urine, saliva, ascites, cerebral spinal fluid and pleural fluid.

11. The method of claim 1, further comprising the step of extracting lipids from the sample of bodily fluid.

20 12. The method of claim 1, further comprising the step of comparing the concentration of phospholipid with an earlier concentration from the same subject.

13. The method of claim 1, wherein an increase or decrease in the concentration of phospholipid relative to normal subjects indicates the presence of the disease condition.

5 14. The method of claim 1, wherein the disease condition is gynecological cancer or ovarian cancer.

15. The method of claim 1, wherein the disease condition is a blood disorder associated with alteration in the level of phospholipid.

ABSTRACT

The present invention is an enzymatic method and diagnostic kits for detecting and
5 quantifying the presence of one or more lysophospholids in a sample of bodily fluid taken from a
test subject. The method uses enzymes in a two step assay and may be used to detect disease
conditions associated with altered levels of lysophospholipids and to correlate such conditions
with altered levels of lysophospholipids.